



TITLE:

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## Short communication

# Behavioral and physiological changes in a juvenile Bornean orangutan after a wildlife rescue



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## ABSTRACT

We report a case of a juvenile female orangutan (*Pongo pygmaeus morio*) that remained in a durian tree (*Durio zibethinus*) for about one month, nearby a road delimiting Danum Valley Conservation Area (DVCA), East Borneo. The juvenile was rescued and brought to Sepilok Orangutan Rehabilitation Centre (SORC). Before rescue and after release, we recorded the juvenile's activity and collected fecal samples for the determination of fecal glucocorticoids (fGC). We compared the behavior and stress levels between the two conditions and made comparisons with DVCA juveniles. Additionally, we obtained hematological and biochemical parameters pre and post-quarantine, to monitor her health condition and recovery. The pre-quarantine diagnosis revealed dehydration, malnourishment and slight anemia, but after quarantine her condition stabilized. The juvenile showed abnormal activity in both conditions, with high bark consumption before rescue and a high proportion of resting time after release. fGC levels were higher in comparison to DVCA juveniles, and showed a marked increase after the release. The results suggest that rescue programs help reestablish health parameters, but release processes are stressful for wild orangutans. Early separation from the mother in orangutans may occur more often than reported, particularly in fragmented habitats, and result in poor health that could hasten death.

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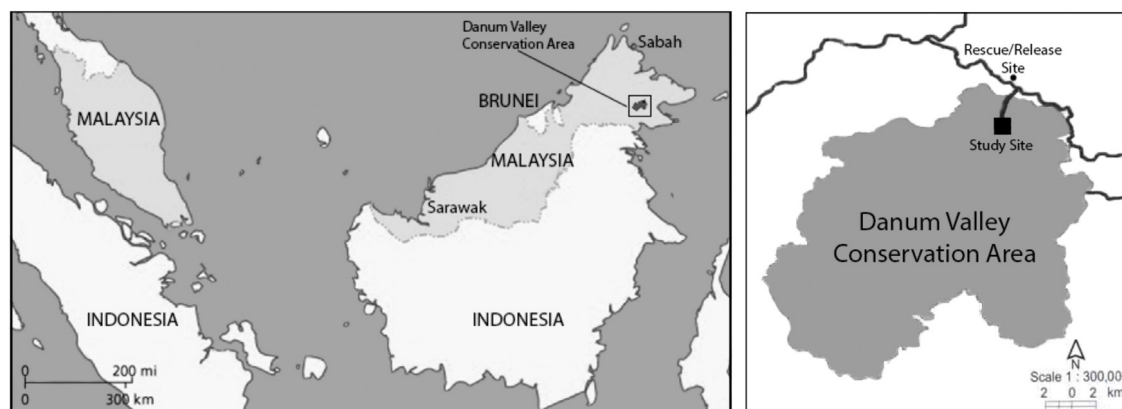
## 1. Introduction

Orangutans have the longest inter-birth interval and exceptionally long developmental phases among the non-human primates (Galdikas and Wood, 1990; van Noordwijk et al., 2009). They attain independence at around 8.8 years old in Sumatra and at around 6.9 years old in Borneo (van Noordwijk et al., 2009). An earlier independence from the mother may expose immature individuals to various threats, such as predation (Kanamori et al., 2012; Rijksen, 1978), harassment from conspecifics (van Noordwijk et al., 2012) and human activities (Meijaard et al., 2011).

According to the IUCN Red List of Threatened Species, both species of orangutans are listed as critically endangered (Borneo, *Pongo pygmaeus*; Sumatra, *Pongo abelli*). Their prolonged developmental period and low birth rate in combination with habitat deforestation (Morrogh-Bernard et al., 2003) and hunting pressure (Marshall et al., 2006), have hampered conservation efforts (Marshall et al., 2009).

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**Fig. 1.** Map of the Danum Valley Conservation Area (left) and the study site, including the location where the juvenile orangutan was rescued/released (right).

Reports on orangutan mortality or disappearance are scarce given their low mortality rate (including infants), when compared to the other great apes, like chimpanzees and gorillas (Wich et al., 2004). Therefore, events that report threatening situations for orangutans are crucial for understanding the possible causes of mortality/disappearance in this species.

Our study is the first to report a case of two wild female juvenile orangutans (*Pongo pygmaeus morio*) isolated in a durian tree outside Danum Valley Conservation Area (DVCA), East Borneo, and the rescue of the youngest female. The two juveniles survived for about one month after the durian fruits finished, with limited access to food resources and the absence of maternal care. For the youngest female, we combined behavioral and hormonal data, hematological, and biochemical analyses to address her relative health status before rescue and after release. Additionally, we compared her behavior and stress levels with wild juvenile orangutans living in DVCA.

## 2. Methods

### 2.1. Study area

The rescue and release of the youngest juvenile female orangutan took place 200 m from the border (consisting of a road) of Danum Valley Conservation Area (DVCA) (05°02.277'N, 117°45.689'E), East Borneo, Sabah, Malaysia. The area consists of a secondary forest, 2 km away from a palm oil concession (Fig. 1). Additionally, we collected data from five dependent immature individuals (infants and juveniles) living in a 2 km<sup>2</sup> area in the DVCA, where studies on orangutans have been ongoing since 2004. DVCA is one of the largest lowland dipterocarp forests remaining in Southeast Asia with a total area of 438 km<sup>2</sup> (Hazebroek et al., 2012) and was established as a Government as a Class I protection Forest Reserve by the Sabah State in 1996. East Borneo is subjected to mast fruiting events that are known to affect orangutan activity, especially their feeding behavior ((Ashton et al., 1988; Wich and Schaik 2000). However, fruit census data collected during the study period showed no mast fruiting event. A small-scale fruiting season occurred in August 2014 (Kanamori et al., in press).

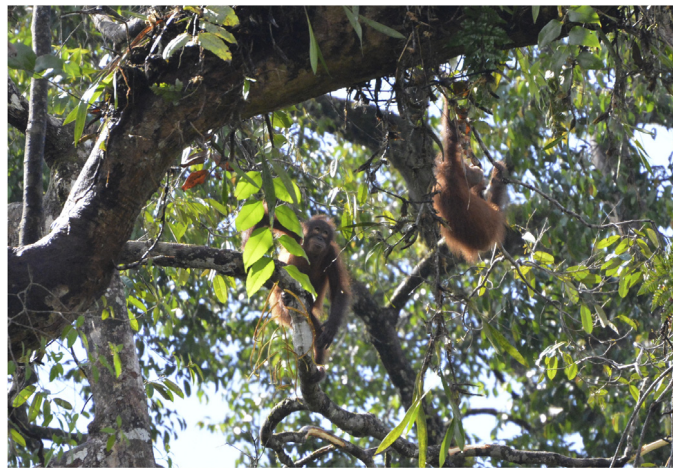
### 2.2. Data collection

RSM and three trained field assistants watched the youngest juvenile female for 33 h 18 min, from 23 to 25 August 2014, in the pre-rescue phase and for 47 h during the post-release phase, from 8 until 12 November 2014. Activity budget data (feeding, resting, traveling) was collected using focal instantaneous sampling every 2 min. Food items consumed by the individual were identified and classified as either low caloric (bark and leaves) or high caloric value (fruits). Fresh fecal samples were collected in the pre-rescue ( $n = 3$ ) and post-release ( $n = 4$ ) phases for the determination of fecal glucocorticoids (fGC). To make comparisons with normal juvenile behavior and fGC levels, we analyzed data from five wild infant and juvenile orangutans (three females and two males) living in DVCA, aging from 3 to 7 years old. Fecal samples ( $n = 11$ ) and behavioral data (276 h) were collected using the same methods described above.

The methods used for collecting data were approved by the Sabah Wildlife Department (SWD) and the Danum Valley Management Committee.

### 2.3. Hormonal assay

All samples were lyophilized using a freeze-drier (EYELA FDU-1200, Tokyo, Japan), pulverized and extracted by adding 5 ml of 80% methanol to 0.1 g of feces. The supernatant was taken to assay the samples after centrifugation ( $100 \text{ G} \times 1$



**Fig. 2.** The two juvenile females in close proximity before the rope being installed.

min). The assay plates were pre-coated with a 100  $\mu$ l/well of Goat Anti-Rabbit IgG (H+L) diluted (1:1400) in a coating buffer (0.01M  $\text{Na}_2\text{CO}_3$ , 0.03M  $\text{NaHCO}_3$ , pH 9.6) and incubated for 2 h at room temperature. Following plate aspiration using an auto-mini plate washer (AMW-8R, Japan), the plate was loaded with a 200  $\mu$ l/well of EIA buffer (0.04M  $\text{Na}_2\text{HPO}_4$ , 0.15M  $\text{NaCl}$ , 0.1% BSA, pH 7.2), and incubated overnight at 4 °C. The plates were washed 2 times (0.05% Tween 80), and an aliquot of 20  $\mu$ l standards, control and samples were added to the designated well in duplicate. Then, 100  $\mu$ l of diluted (1:4000) Rabbit Anti-Cortisol-3-CMO (FKA 404-E, Cosmo Bio Co. Ltda, Tokyo, Japan) and 100  $\mu$ l of diluted (1:3200) Cortisol-3-CMO-Horseradish peroxidase (FKA 403, Cosmo Bio Co. Ltda, Tokyo, Japan) were added to each well and incubated overnight at 4 °C. After washing the plates 4 times, a 150  $\mu$ l/well of substrate (0.01M urea hydrogen peroxidase, 0.1M  $\text{Na}_2\text{HPO}_4$ , 0.05M citric acid, 0.002M tetramethylbenzidine, 4% dimethylsulfoxide) was added, and the plates were incubated in the dark at 37 °C for 10 min. Then, 50  $\mu$ l/well of stop solution (6.45N  $\text{H}_2\text{SO}_4$ ) was added and the plates were read at 450 nm (TECAN Rainbow Sunrise, Grödig, Austria). To validate the assay, we collected daily samples ( $n = 6$ ) from a female orangutan at the OrangUtan Island (OUI, Semmangol) that was submitted to a stressful procedure of anesthesia and blood sampling for a routine health check-up. We observed a peak on fGC levels one day after the procedure (supplement Fig. 1, Appendix A), which indicates that the assay detected a fecal glucocorticoids response. Assay cross-reactivities were 100% for cortisol (Compd. F), 11.5% for 11-Deoxycortisol (Compd. S), 4% for cortisone (Compd. E), 2% for corticosterone (Compd. B), 0.2% for 17 $\alpha$ -Hydroxy-11-Deoxy-Corticosterone (Compd. A), 0.04% for 17 $\alpha$ -Hydroxy-Progesterone, and 0.0% for other steroids. The sensitivity of the assay was 0.2 ng/ml. The mean intra-assay coefficient of variance was 5.77% ( $n = 24$ ) and the inter-assay coefficient of variance was 6.56% ( $n = 2$ ).

#### 2.4. Biochemistry and Hematology

Blood collection for hematological and biochemistry analysis was performed by the veterinarians and staff of SORC using established clinical tests that were analyzed at Gribbles Pathology laboratory in Selangor, Malaysia. The parameters analyzed are described in Table 1. Reference values provided from the laboratory are from humans. Because some orangutan's references available in the literature are not discriminated by age or gender (Kilbourn et al., 2003, Schmidt et al., 2006; but see: McClure et al., 1972), we have also added reference values for chimpanzees as a comparison (Ihrig et al., 2001).

### 3. Results and discussion

The incident occurred in a secondary forest 200 m from a main road. Several orangutans were reported to visit a durian tree (*Durio zibethinus*) from the end of June through July (when the durian fruits finished). We first observed the two juvenile females in the end of July (Fig. 2). We were able to confirm around the third week of August that two juvenile females were unable to leave the tree and had been stranded for at least one month through anecdotal observations by RSM and local guides. The only connection to an adjacent tree had been broken, preventing the two juveniles from moving to another tree. Because of their smaller body size in comparison to adult individuals, the juveniles could not use their weight to swing the branch and reach adjacent tree branches. Therefore, in order to rescue the two juvenile females, we contacted the Wildlife Rescue Unit (WRU) of SWD. They installed a rope that was used by the older juvenile female (estimated age of 7 years) to move down on the following morning (Fig. 3). The younger juvenile female (estimated age of 4–5 years old) did not show any interest in the rope, and after five days she was rescued by WRU and taken to SORC for a quarantine period of 75 days. After the end of the quarantine period, the younger juvenile female was released at the point of rescue.



**Table 1**  
Results of clinical analysis on biochemistry and hematology.

Elements (SI Units)	After rescue	Before release	Reference values (humans)	Reference values (juvenile female chimpanzees) <sup>a</sup>	Reference values (orangutans) <sup>c</sup>
<b>Lipids</b>					
Total cholesterol (mmol/L)	4.2	4.8	<5.2	4.63–7.55	2.25–6.10 <sup>d</sup>
Triglyceride (mmol/L)	0.37	0.81	<1.68	0.40–1.34	0.42–2.04 <sup>d</sup>
HDL cholesterol (mmol/L)	2.24	1.94	<1.03	1.09–2.69	0.39–2.12 <sup>d</sup>
LDL cholesterol (mmol/L)	1.79	2.49	<2.58	2.95–5.33	0.98–3.6 <sup>d</sup>
<b>Electrolytes</b>					
Potassium (mmol/L)	6.3	5.4	3.5–5.1	3.29–4.33 <sup>b</sup>	3–8.9 <sup>e</sup>
Chloride (mmol/L)	72	99	95–110	94–113 <sup>b</sup>	11–118 <sup>e</sup>
Sodium (mmol/L)	140	141	138–145	136.57–145.30 <sup>b</sup>	34–157 <sup>e</sup>
<b>Renal function</b>					
Urea (mmol/L)	9.6	1.1	2.5–8.0	3.68–6.53	–
Creatinine (umol/L)	79	42	50–110	44.2–75.14 <sup>b</sup>	61.88–972.4 <sup>e</sup>
Uric Acid (mmol/L)	0.55	0.2	0.15–0.45	0.95–3.6 <sup>b</sup>	–
Calcium (mmol/L)	2.33	2.34	2.10–2.55	2.04–2.63 <sup>b</sup>	0.5–2.7 <sup>e</sup>
Phosphate (mmol/L)	4.59	2.04	0.65–1.45	–	–
<b>Liver function</b>					
Total protein (g/L)	80	72	55–74	63–77.10	55–85 <sup>e</sup>
Albumin (g/L)	50	40	33–47	32.9–41.8	29–56 <sup>e</sup>
Globulin (g/L)	30	32	20–39	26.6–38.4	20–35 <sup>e</sup>
Alkaline phosphatase (U/L)	301	714	30–120	354.7–658.23	2–1186 <sup>e</sup>
Aspartate aminotransferase (U/L)	52	41	<41	12.94–26.47	13–2844 <sup>e</sup>
Alanine aminotransferase (U/L)	59	44	<51	28.24–47.65	14–520 <sup>e</sup>
<b>Diabetic screen</b>					
Glucose (mmol/g)	0.2	4.7	3.9–5.55	3.68–6.53	3.17–15.28 <sup>e</sup>
<b>Hematology</b>					
Hemoglobin (g/L)	102	102	100–140	120.5–146	103–150 <sup>f</sup>
MCHC (g/L)	278	268	300–360	320–343	282–341 <sup>f</sup>
White Cell Count (x 10 <sup>9</sup> /L)	12.9	11.2	5.0–12.0	8.3–43.33	7.2–32 <sup>f</sup>
Neutrophils (/L)	7.6	5	2.0–6.0	2.48–11.41	1.98–27.2 <sup>f</sup>
Lymphocytes (/L)	4.6	5.4	1.0–4.0	2.31–7.20	0.47–20.9 <sup>f</sup>
Platelets	176	175	150–400	225–413.05	144–480 <sup>f</sup>

<sup>a</sup> Ihrig et al. (2001).

<sup>b</sup> Reference values for adult females, as they are not available for juvenile females.

<sup>c</sup> Orangutan references are taken from different sources:

<sup>d</sup> Schmidt et al. (2006).

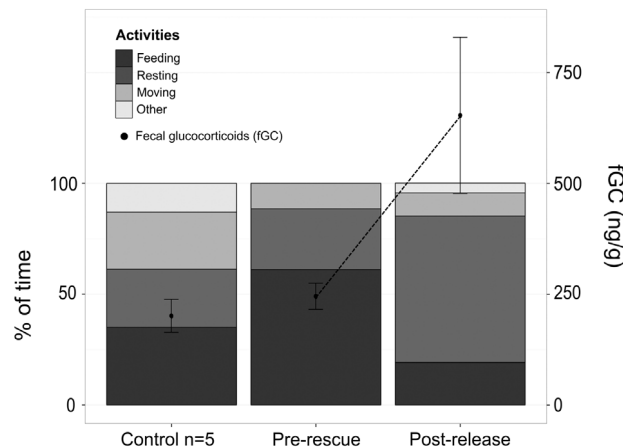
<sup>e</sup> Kilbourn et al. (2003).

<sup>f</sup> McClure et al. (1972).



**Fig. 3.** Older juvenile female in the lowest branch of the tree looking down (left). Older juvenile female approaching the rope after being installed (right).

Female orangutans are philopatric and establish home ranges that overlap or are proximate to their mothers' (Goossens et al., 2006; Nietlisbach et al., 2012; van Noordwijk et al., 2012). Releasing the orangutan at the point of rescue increases



**Fig. 4.** Activity budget and fGC levels (mean±SE) of the juvenile in the pre-rescue and post-release conditions and juveniles from DVCA (control).

the chances of her meeting the mother and continuing the complex process of acquiring the necessary skills for surviving independently (van Noordwijk et al., 2009). However, given the estimated age of the younger female (4–5 years old) and considering that Bornean orangutans become independent at the age of 6–7 years (van Noordwijk et al., 2009), we assumed that her mother may have abandoned her or died.

During our observations in the pre-rescue phase, the juvenile female's feeding time was higher in comparison to the wild juvenile orangutans inhabiting DVCA (Fig. 4). In the pre-rescue phase, her diet consisted almost entirely of low quality items: 51% bark, 48% of leaves, as the durian tree had finished fruiting. Orangutans are mainly frugivorous, and fruits comprise more than half of their diet (Russon et al., 2009). For one month, the juvenile female's diet was deprived of fruits and her diet consisted only of low quality items. Orangutans do not usually increase feeding time during the fallback season, when the consumption of low caloric items is higher (Morrogh-Bernard et al., 2009; Kanamori et al., 2010). Instead, they adopt a “sit and wait” strategy to conserve energy by increasing the resting time, a pattern observed mainly in Bornean populations living in dipterocarp forests (Morrogh-Bernard et al., 2009). Additionally, as Bornean orangutans experience higher fluctuations in fruit availability and longer seasons of fruit shortage (Kanamori et al., 2010; Morrogh-Bernard et al., 2009) they may be particularly adapted for fat storage (Knott, 1998). This metabolic adaptation allows them to survive on low quality food in seasons of low fruit availability. In this particular case, the high proportion of feeding time in the pre-rescue phase, potentially reflected her need to compensate the low caloric diet as a consequence of a very limited ranging area and feeding choices.

In the post-release phase, the activity budget of the juvenile female showed a high proportion of resting and a low proportion of feeding time, of which 11% consisted of fruits. Bark ingestion dropped substantially and the ingestion of leaves increased to 88%. In order to better interpret our results, we would have needed to conduct phenological measurements in conjunction with observations of orangutans living in the same area. However, because this was an isolated event outside of our study site, we could not determine the fruit availability and feeding behavior of individuals living in that area. Therefore, the possibility of a post-captivity response should also be considered as an explanation for her decreased feeding time during the post-release phase. After being released, the juvenile female climbed up a 50 m tree, and spent three nights there. Young orangutans are reported to practice nest building in order to acquire the necessary skills to survive after independence (van Noordwijk and van Schaik, 2005; van Adrichem et al., 2006; van Noordwijk et al., 2009). However, in the post-release phase, the time she spent building the nest (11% her active time), was higher than other infants and juveniles from DVCA ( $\leq 1\%$ ). This may reflect her need for practicing this behavior after remaining in captivity for more than two months. By contrast, it could have been that her behavior was abnormal because of being stranded in a tree, and later isolated in captivity. However, as the days passed, her activity budget gradually started to resemble that of her wild immature counterparts, suggesting that she was gradually readjusting her behavior to the environment.

Results from hematological and biochemical analysis support the behavioral observations. Before the rescue, increased total protein levels, the activity of the enzymes AST and ALT, and the extremely low levels of glucose (Table 1), suggest that the orangutan was malnourished and suffered from liver malfunction. However, despite the ingestion of low quality food for 1 month, her condition was not yet so severe. Biochemistry analysis also showed signs of dehydration and a mild kidney malfunction before rescue, reflected by the elevated urea phosphate, creatinine and uric acid levels (Table 1).

After quarantine, the renal and hepatic functions also seemed to return to a normal range of values, except Alkaline Phosphatase (ALP) which had more than a two-fold increase in plasma levels. However, given the age of the orangutan, ALP could be high and unstable because of the exacerbated osteoblastic activity in growing individuals (Schmid and Forstner, 1986; Takeshita et al., 2011; Turan et al., 2011). The juvenile female was also diagnosed as having anemia hypochromic, before and after quarantine, which is mainly caused by iron deficiency (Cook, 1990). Iron supplementation may take a few months to replenish hemoglobin in the blood (von Drygalski and Adamson, 2012), which could account for why

hematological tests did not change after quarantine. However, during the quarantine, the juvenile was re-hydrated and gained enough weight (8–12 kg) to return to the wild where she can have access to her regular food supply and recover slowly from anemia without undergoing the stress of captivity for a longer period.

The hormonal assay results revealed that fGC levels (242 ng/g) were slightly higher than the control ( $198 \pm 37.25$  ng/g), which suggests that the orangutan was under chronic stress after 1 month in the tree, corroborating with the behavioral data and the results of the clinical analyses. fGC concentrations increased drastically after release (650 ng/g, Fig. 4), indicating acute stress levels. Previous studies in rhesus (*Macaca mulatta*) (Schapiro et al., 1993) and Japanese macaques (*Macaca fuscata*) (Takeshita et al., 2014) showed an acute increase of cortisol levels when undergoing a sudden change in their environmental conditions. The increased fGC concentrations could also be a consequence of the long transportation process (Weingrill et al., 2011), given that the orangutan traveled around 220 km before reaching the release site. After a few days, cortisol should return to baseline levels as a reflection of the adjustment to a new environment (Behringer et al., 2014), however, we lack substantial data to confirm this trend.

For orangutans, arboreality may be a good adaptive strategy against predation (Shattuck and Williams, 2010), but young individuals may be more vulnerable when subjected to an early separation from the mother. This is exacerbated in damaged habitats, as our study reports. In combination, behavioral, hormonal and clinical analyses support that the juvenile female showed signs of stress, malnutrition, dehydration and anemia. Levels were not so severe, but for a prolonged period of time it could have potentially been fatal. These signs are indicators of bad health caused by isolation, absence from the mother and consumption of low quality food for about one month. To assure the survival of the individual, we proceeded with the rescue and subsequent release of the individual.

Our study suggests that young orangutans adjust their behavior and physiology to adverse environmental conditions to a certain extent, but human involvement may be required to ensure their survival. Despite conservation efforts, both species of orangutans are still experiencing a marked decline, largely due to human activities. Identifying life threatening situations and evaluating the benefits and potential risks of rescue and release processes, as well as monitoring the behavior and the health condition of the orangutan, is essential to attenuate the serious threats which this endangered species is currently confronting.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.gecco.2016.08.004>.

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